Consideration of the problem of pollution of water with viruses requires that a minimal infective dose of viruses by the oral route be established. This presentation describes a study of low doses of attenuated live poliovirus administered to infants and points out that any dose sufficient to infect tissue culture would also be sufficient to infect man.

MINIMAL INFECTIVE DOSE OF ATTENUATED POLIOVIRUS FOR MAN

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Introduction

In studies of experimental infection with poliovirus in man, large doses have been deliberately used in order that an infection rate approaching 100 per cent be achieved. Therefore the lower limit of infectivity has rarely been established. Yet consideration of such a limit raises important theoretical and practical points.

First, doses of virus administered to experimental animals and man are customarily expressed in terms of their infectivity in an in vitro system. Thus the dose of attenuated poliovirus is usually expressed as a multiple of the quantity producing infection in 50 per cent of monkey kidney tissue cultures exposed to the virus. However, the relationship between infectivity of poliovirus for tissue culture and for man has never been assessed quantitatively.

Second, considerations of environmental contamination of water supply or food with viruses demand that a minimal infective dose of these agents for man be established, so that standards for pollution control may be determined.

Attenuated poliovirus infection in man lends itself well to the study of dosage since tissue culture titration systems and methods of administration are well established.

Data obtained in several past experiments provide some information about minimal doses of attenuated polioviruses for man. The principal relevant study is that of Koprowski,[1,2] who administered the SM strain of the poliovirus Type I in gelatin capsules to human volunteers. He showed that 2 PFU produced infection in two out of three subjects, while 0.2 PFU did not infect three others (Table 1). In another study Koprowski, et al.,[3] succeeded in infecting one subject with the SM strain using 20 PFU and three subjects using 200 PFU. The P712 strain used by Sabin[4] was infective in several volunteers at the dose of 100 TCD_{50}. Plotkin, et al.,[5] using the Fox strain of poliovirus Type III, showed that doses of 30 to 80 TCD_{50} infected seven of nine subjects.

The present experiment was designed to test low doses of the Fox strain of poliovirus Type III for their infectivity for man.

Table 1—Infection with attenuated poliovirus Type I (SM strain) of adult volunteers

Dose (PFU)	Result (carrier rate)	% infected
200	4/4	100
20	4/4	100
2	2/3	67
0.2	0/2	0

Methods

The subjects were 22 premature infants in the nurseries of the Philadelphia General Hospital, whose weights ranged from 1,500 gm to 2,200 gm. Each was given a calculated dose of the Fox strain of Type III poliovirus within the first 48 hours of life. The vaccine was administered by gavage in 5 cc of Hanks' solution using a rubber oro-gastric tube, into whose wide distal opening the virus solution was decanted. The tube was flushed was 10 cc of saline before its removal. Each infant was observed for 15 minutes to insure that there was no regurgitation. One prevaccination stool specimen was collected. Postvaccination stool specimens were obtained on the third day following the administration of the virus and at three-day intervals until a total of four specimens were collected. Ten per cent stool suspensions were made in Hanks' solution and 0.1 ml aliquots of each stool suspension were then inoculated into four primary African green monkey kidney tissue culture tubes obtained commercially. Inoculation was carried out by decanting the medium and incubating the tubes with the stool suspension for 30 minutes, at 37° C. Following adsorption, the tubes were washed with PBS solution and incubated for one week in Eagle's medium enriched with 2 per cent fetal calf serum. They were then examined for the presence of cytopathic effect. All specimens which showed CPE were passaged once. Virus was harvested and identified by neutralization with serum of a rabbit immune to Type III poliovirus. All negative specimens were blindpassaged once.

Three concentrations of the vaccine virus, 10 TCD_{50}, 2.5 TCD_{50}, 1 TCD_{50} were prepared as follows. The concentration of 10 TCD_{50} was prepared by the appropriate dilution of the stock virus. The two lower doses were prepared in like fashion, but before they were administered each was retitrated by inoculation of 0.1 ml aliquots into 50 GMK tubes.

Results

The suspension of virus containing presumptively 2.5 TCD_{50} per ml gave CPE in six out of 50 tubes inoculated with 0.1 ml aliquot. The predicted number was 6.25 tubes. For the 1 TCD_{50} per ml solution, the observed number was two out of 50 tubes as compared with the predicted number of 2.5.

Two of three infants given 10 TCD_{50} of the virus became infected. In the two groups receiving lower doses, three of nine infants given 2.5 TCD_{50} of the virus and three of 10 infants given 1 TCD_{50} of the virus became infected (Table 2).

Statistical Analysis

The results were plotted on log-probability paper and a line was fitted to the

Table 2—Infection with attenuated poliovirus Type III (Fox strain) of premature infants

Dose (TCD_{50})	Result (carrier rate)	% infected
10	2/3	67
2.5	3/9	33
1	3/10	30

points using chi-square analysis. This line could be described by the formula: $\log A = \log 3.5 + 0.023 \times (B - 47\%)$, where A is the virus dose in TCD_{50} and B is the per cent of subjects infected.

The probable 50 per cent infective dose for infants was thus calculated to be 4 TCD_{50}. It could also be predicted that 10 per cent infection in premature infants would result from administration of 0.3 TCD_{50}.

Discussion

The term "minimal dose" requires elaboration. If one states that a virus has a concentration of, for example, 10 TCD_{50}, one means that the suspension, when diluted tenfold, would infect 50 per cent of the tissue culture. Obviously, when diluted a hundredfold, it would on strictly mathematical grounds infect one of 20 cultures.

In this experiment the amount of poliovirus actually fed was carefully established in terms of infectivity for susceptible tissue cultures. Newborn premature infants are known to be highly susceptible to attenuated virus infection.[6] Cord blood antibody titers were not determined, but if maternal antibody had any influence on our subjects, it would be to decrease their susceptibility to the virus. The attenuated virus used is relatively infective for man[5] and its delivery directly into the stomach by gavage tube precluded any inaccuracy due to loss in the mouth or regurgitation.

Our results show that 30 per cent of subjects given enough virus to infect 50 per cent of tissue cultures—i.e., 1 TCD_{50}—were infected. The calculated 50 per cent infective dose for these infants was 4 TCD_{50}, but this figure may have been influenced by the apparent resistance of some newborn infants to any dose of attenuated poliovirus.[7] It seems reasonable to assume that virulent polioviruses and other wild enteroviruses are at least as infective as the attenuated virus we used.

The potential effect of even a small degree of water and food pollution by virulent viruses may be greater than has been generally realized. Contamination with a small quantity of a potentially pathogenic virus may be of great consequence for some individuals, even if the proportion of affected individuals in the community remains quite low.

In a recent review of the available evidence,[8] we advanced the concept that a dose of any pathogenic virus sufficient to infect tissue culture would also be infective for man. These results seem to support that view.

Summary

The problem of assessment of water pollution with viruses demands knowledge of minimal infective doses of viruses by the oral route. Attenuated live oral poliovirus vaccine was used as the test material and 22 infants served as subjects. They were divided into three groups and received 10, 2.5, and 1 tissue culture doses of the virus respectively. The virus was infective even at the lowest dilution. The results tend to support the contention that any dose of virus sufficient to infect tissue culture would also be infective for man.

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Health Care Goals

In this nation today we have begun to explore energetically, seriously, sympathetically, the conditions—all the conditions—that prevent individual fulfillment. Through all of these efforts—whether they involve health or racial justice or education or the attack on poverty—run the same great themes: the release of human potential, the enhancement of human dignity, the liberation of human spirit.

(John W. Gardner. "Health Care Goals for the Great Society." New York Univ. Med. Quart. 22,4:10 (Spring), 1967.)